



NAVIGATIONAL STRATEGIES OF MIGRATING MONARCH BUTTERFLIES

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Final Report

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FINAL PROGRESS SUMMARY

To: technicalreports@afosr.af.mil

Subject: Final Progress Statement to (Dr. Patrick Bradshaw)

Contract/Grant Title: Navigational Strategies of Migrating Monarch Butterflies

Contract/Grant #: FA9550-10-1-0480

Reporting Period: 01-Sept-10 to 31-Aug-14

Overview of accomplishments:

Migrating monarch butterflies (*Danaus plexippus*) use a time-compensated sun compass to navigate from eastern North America to their overwintering grounds in central Mexico. We have described the neuronal layout of those aspects of the butterfly brain central complex likely to establish part of the internal sun compass and find them highly homologous to those of the desert locust (1, 2). Intracellular recordings from neurons in the monarch sun compass network reveal responses tuned to specific *E*-vector angles of polarized light, as well as azimuth-dependent responses to unpolarized light, independent of spectral composition (1). The neural responses to these two stimuli in individual neurons are mediated through different regions of the compound eye. Moreover, these dual responses are integrated to create a consistent representation of skylight cues in the sun compass throughout the day. The results advance our understanding of how ambiguous sensory signals are processed by the brain to elicit a robust behavioral response.

The sun compass timing elements reside in light-entrained circadian clocks in the antennae. We have shown that either antenna is sufficient for proper time compensation (3). However, migrants with either antenna painted black (to block light entrainment) and the other painted clear (to permit light entrainment) display disoriented group flight. Remarkably, when the black-painted antenna is removed, re-flown migrants with a single, clear-painted antenna exhibit proper orientation behavior. Molecular correlates of clock function reveal that *period* and *timeless* clock gene expression is highly rhythmic in brains and clear-painted antennae, while rhythmic clock gene expression is disrupted in black-painted antennae. Our work shows that clock outputs from each antenna are processed and integrated together in the monarch time-compensated sun compass circuit. This dual timing system is a novel example of the regulation of a brain-driven behavior by paired organs.

We also generated a standardized average-shape representation of the central complex and related brain regions known to be involved in processing skylight compass cues (4). This tool was used to register individual neurons into a common frame of reference. We identified central complex input pathways, intrinsic neurons, and output pathways. Interspecies comparisons suggest a conserved ground pattern of central complex neuroarchitecture.

In the spring, the same migrants that have overwintered in Mexico remigrate northward to the southern United States to initiate the northern leg of the migration cycle. We found that spring remigrants also use an antenna-dependent time-compensated sun compass to direct their northward flight (5). Fall migrants premature exposed to overwintering-like coldness reverse their flight orientation to the north. The temperature microenvironment at the overwintering site is essential for successful completion of the migration cycle, because without cold exposure, aged migrants continue to orient south. The discovery that coldness triggers the northward flight direction in spring remigrants underscores how vulnerable the migration may be to global warming and overall global climate change.

Using flight simulator studies, we recently showed that migrants possess an inclination magnetic compass to help direct their flight equatorward in the fall (6). The use of this inclination compass is light-dependent utilizing ultraviolet-A/blue light between 380 and 420 nm. Notably, the significance of light <420 nm for inclination compass function was not considered in previous monarch studies. The antennae are important for the inclination compass because they appear to contain light-sensitive magnetosensors. For migratory monarchs, the inclination compass may serve as an important orientation mechanism when directional daylight cues are unavailable and may also augment time-compensated sun compass orientation for appropriate directionality throughout the migration. Knowledge of the presumed location of the relevant light-sensitive component of the magnetosensor in monarchs opens the way for evaluation of both the molecular and genetic mechanisms of magnetoreception.

A synthesis of our current understanding of navigational mechanisms in migrating insects has been presented (7).

Archival publications (published) during reporting period:

1. Heinze S and Reppert SM (2011) Sun compass integration of skylight cues in migratory monarch butterflies. **Neuron** 69: 345-358.
2. Heinze S, Reppert SM (2012). Anatomical basis of sun compass navigation I: The general layout of the monarch butterfly brain. **J Comp Neurol** 520:1599-1628.
3. Guerra PA, Merlin C, Gegear RJ, Reppert SM (2012). Discordant timing between antennae disrupts sun compass orientation in migratory monarch butterflies. **Nat Commun** 3:958.
4. Heinze S, Florman J, Asokaraj S, el Jundi B, Reppert SM (2013). Anatomical basis of sun compass navigation II: The neuronal composition of the central complex of the monarch butterfly. **J Comp Neurol** 521:267-298.

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5. Guerra PA, Reppert SM (2013). Coldness triggers northward flight in remigrant monarch butterflies. ***Curr Biol*** 23:419-423.
6. Guerra PA, Gegear RJ, Reppert SM (2014). A magnetic compass aids monarch butterfly migration. ***Nat Commun*** 5:4164.
7. Merlin C, Heinze S, Reppert SM (2012). Unraveling navigational mechanisms in migratory insects. ***Curr Opin Neurobiol*** 22:353-361.

Changes in research objectives, if any: None

Change in AFOSR program manager, if any: Over course of the grant a change from Dr. Willard Larkin to Dr. Patrick Bradshaw

Extensions granted or milestones slipped, if any: None

Include any new discoveries, inventions, or patent disclosures during this reporting period (if none, report none): None

1. We discovered that the central complex, a mid-line brain structure, functions as the sun compass in migrant monarchs. Eye-sensed skylight cues (solar azimuth and polarized light) are dually integrated in the central complex and associated regions to provide an internal representation of skylight cues for directionality. The results add to our understanding of how ambiguous sensory signals are processed by the brain to elicit a robust behavioral response.
2. We discovered that the thermal microenvironment that migrant monarchs are exposed to at the overwintering sites is essential for the directional switch in sun compass orientation in spring re-migrants. Knowledge of the “cold trigger” in the migration of the monarch butterfly has broad-ranging implications for conservation strategies related to this endangered biological phenomenon and underscores how vulnerable the migration may be to global warming and overall global climate change. Furthermore, we can now dissect the molecular mechanism by which low temperature causes the switch in flight direction and the location of the relevant temperature sensor.
3. We discovered the existence of an inclination magnetic compass in migrant monarch butterflies. This discovery provides a valuable experimental paradigm in which the mechanisms of bio-magnetic sensing can be studied using anatomical, cellular, molecular, and genetic approaches.